

MICROORGANISMS IN RELATION TO THE PRODUCTION AND DEGRADATION OF SOIL ORGANIC MATTER

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IN dealing with this subject in the time available, only a few of the chief aspects of the subject can be touched upon, otherwise a mere catalogue of microorganisms and of chemical compounds would result.

In the first place, it is well to consider shortly what we have in mind as soil organic matter, and the general problems which its chemistry involves. In recent years, the use of the terminology which had developed historically has become obscured largely as a result of the impact of microbiology on earlier chemical concepts. Older chemists recognized as characteristic of the organic matter of soil the dark-coloured complex, called humus, which was in various ways bound up with soil fertility, which they regarded as the more stable end-point of the decomposition of such organic residues as were incorporated in the soil system. This consisted of finely-divided colloidal matter, seemed to be more or less separable from the rest of the soil by suitable extractants, such as alkalis, and could be subdivided by further treatment into fractions which had sufficient identity to be given names such as fulvic acid, humatomelanin acid, humic acid, etc. With few exceptions these workers recognized at an early stage that these substances were not chemical entities any more than, for example, such names as pyroligneous acid, although each name was considered to have a distinct significance as indicating a complex of similar or homologous groups. The separation and identification of these was beyond the power of chemists of the period.

The use of the word humus, by Waksman and his followers, to cover the whole of soil organic matter seems to have arisen at least in part as a result of the sense of frustration which this position induced, and in part to the difficulty of connecting the older chemistry with the new microbiology and biochemistry which were at first directed toward the study of the decomposition of plant residues in the soil. The later method of study usually called the proximate analysis of soil, with which Waksman's name is usually associated, is an attempt to apply somewhat more rationalized chemical methods to the problem of the relationship which exists between plant decomposition and the organic matter found in soil. But it provides only a general balance sheet of the changes which take place in decomposing organic matter, and like many balance sheets may throw little light on the mechanism of the changes involved. It may give no indication of any elaboration of organic matter which takes place alongside decomposition. In fact, it classifies together the living and dead organic matter and when it is realised that in an eutrophic, moderately dry, soil over 30% of the organic matter may be microorganic—living and dead—the approximate nature of such general summations is apparent. The method of Springer in which the living and undecomposed residues are considered to be separated from the decomposed by treatment with acetyl bromide is scarcely likely to be less approximate, and can be useful only as a routine procedure for differentiating soil types on a statistical basis.

It is now becoming generally recognized that these methods of mass investigation have reached a stage at which the accumulation of further data is of doubtful value, and it becomes apparent that further progress in the study of soil organic matter is more likely to be made from the microbiological side than from the application of direct chemical analyses to soil organic

matter in the mass. This on the chemical side is a reversion in some respects to the aims of Schreiner and Shorey during the last century, to isolate actual chemical constituents from the soil, but is being extended to include the activities of those organisms which produce them—whether by their catabolic or by their anabolic functions.

I propose, therefore, to review some aspects of the progress which has already been made in this direction in the past and to indicate some of the general problems involved in such investigation of soil organic matter. I shall confine myself to consideration of the major constituents of soil organic matter, neglecting those trace constituents in which much of the most striking progress has been made in recent years. I refer, of course, to such substances as vitamins, growth hormones, toxins or growth inhibitors, the presence of which in soil, however important, does not contribute to any extent to the mass of soil organic matter as a whole.

The major substances to be considered can be grouped under the general headings of fats and waxes, carbohydrates, nitrogenous compounds, and aromatic compounds; and the relationships of soil microorganisms to these will be considered in that order.

Fats and waxes

The alcohol-benzene solubles usually thus designated consist of two principal groups, the fats and fat-like lipid compounds and the waxes which include not only sterols, but also higher chain hydroxy acids and alcohols. The data on the decomposition of these do not provide much of special interest. Glycerides are apparently decomposed by members of most groups of microorganisms—with the liberation of fatty acids. The higher acids, like the higher carbohydrates, are not apparently generally available to microorganisms. Reference, however, should be made to the fact that Actinomycetes, especially the Proactinomyces, do attack these, and it appears from the work of Jensen¹ that some of the Proactinomyces which seem to be unable to utilize carbohydrates such as cellulose, obtain their energy freely from solid paraffinic hydrocarbons. Further information is required on this question, since long chain paraffinic compounds are produced not only by most groups of microorganisms, but also by a wide range of higher plants. The combined wax and fat content of bacteria associated with soil may amount to 10% or more of the dry weight, although usually much lower. That of the smaller fungi, such as yeasts, may be greater than 10%.

In mass, fats and waxes accumulate in soils such as rich horticultural soil, to which large amounts of organic manures are added, and are considered to have an unfavourable effect on soil structure. And again, in oligotrophic, acid soils such as heath and moorland in this country, where microbial activity is low and where higher plants produce considerable amounts of protective hydrocarbons, the wax content of the humified organic matter can be quite high—up to 20% of the total carbon—indicating that in such soils waxes form one of the major accumulating constituents of soil organic matter.

Without examination of the actual constituents of these waxes, and of the waxes produced by relevant soil organisms, it is impossible to assess the respective parts played in humification by degradation and synthesis in these wax accumulations.

Carbohydrates

In the past, soil workers have been interested in carbohydrate metabolism in two principal directions, namely, the decomposition of cellulose and the utilization of sugars and perhaps somewhat more complex carbohydrates as growth media. In spite of the large volume of work carried out on cellulose decomposition, very little is known as to the details of this in soil, although it is likely that cellobiose is formed as a first stage with some bacteria, and that glucose is likely made available at some stages for other organisms. It is certain also that some of the common cellulose decomposing organisms produce bacterial gums and so may be considered as maintaining the carbohydrate content of the soil by utilizing cellulose in part.

When plant material is added to the soil it appears that much of the simpler carbohydrates, sugars, pentosans and the general group of hemi-celluloses is fairly rapidly attacked—especially if sufficient nitrogen and phosphate are available. The decomposition at this phase seems to be mainly brought about by bacteria. (In composting, it is this phase of intense activity which produces heat.) Subsequently fungi appear to play a more active part than bacteria, at least in the ordinary natural soils of the north of Scotland which are oligotrophic or at most mesotrophic types. This may be true, therefore, of the majority of the heath-coniferous forest types of the cold temperate region, although in artificial culture-soils of the same region it is considered that bacteria may be predominantly active. (It may be mentioned that in our composting experiments the degradation of cellulose is a slower process and takes place only after the thermal phase is over. This is mentioned because of the emphasis laid on thermophiles in the investigations of digestive bacteria in the rumen of herbivores.)

Turning to the synthesis of carbohydrates by microorganisms—although the evidence is not exhaustive it seems that true cellulose is produced by many microorganisms. The well-known membranes produced by *Azotobacter xylinum* consist of some 50% of cellulose. Along with such cellulose structures are usually found hemi-celluloses and in fungi especially chitin is frequent. It is difficult to bring to any satisfactory system the apparently contradictory evidence of different workers.

At the present time one of the most interesting and rapidly advancing subjects in microbiology is the investigation of those less highly polymerized carbohydrates produced by microorganisms generally called bacterial gums or mucilages. Although the interest in these in general microbiology lies in their serological properties, the pathological work has greatly stimulated interest in the subject among soil bacteriologists. For some time it has been recognized that polyuronides are present in small but varying amounts in soil as determined by the usual method of decarboxylation by 12% hydrochloric acid. Various workers (Norman,² Tiurin,³ Waksman,⁴ for example) have compiled data on the subject. Shewan⁵ (1937) estimated that in one example, the B horizon of a sandy podzol from Aberdeenshire, some 30% of the carbon was uronic. Later (in 1946), Forsyth⁶ isolated from a series of soil types a uronide gum or complex of gums which was considered to be identical for all the soils investigated. This work has been followed up by Forsyth and Webley⁷ at the Macaulay Institute, in which they find that of the bacteria plated out from four representative but ecologically distinct soil types, some 11% are gum-producers. Of those bacteria investigated, 38% produce levans; 45% produce glucose-uronics; 10% produce glucose-mannose-uronics; 12% produce glucose-mannose-rhamnose-uronics. Some of these produced more than one type of mucilage. More complex types of polyuronide than those have been found by other workers, although glucose is commonly the most abundant monosaccharide present. These substances do not usually accumulate in the soil to any considerable extent—from 0.2%

to 2% of the total organic matter in the upper horizon of the soil, but, in our region, there is a definite correlation between the natural fertility of soil and uronide content at the surface. Since such polysaccharides are apparently readily attacked by other microorganisms, it is likely that the continuous synthesis and decomposition of such substances is of considerable importance in the general economy of the soil. The water-holding power of soils may be directly affected by them, and it is interesting to note that Russian scientists,⁸ working on the effect of organic matter on soil structure, maintain that it is not what is added to the soil that improves soil structure, but the mode of decomposition of the material. A process of metabolism such as this, in intimate contact with the mineral particles of the soil, provides a satisfactory mechanism for the formation of crumb structure just as does the microbial synthesis of humic material which will be referred to later.

Nitrogen compounds

For plant nutrition the nitrogenous compounds are the most important constituents of the soil organic matter, and are of all plant nutrients the most difficult to assess the immediate value or availability to growing plants. This is, of course, to be expected because of their variety in molecular structure and in susceptibility to change. In general terms, their transformations have now been long established and little new has been added to our knowledge in recent years. We may, however, note that the availability of nitrogen to plants as a whole is bound up with the general biotic or microbiological activity of the soil. For example, additions of carbohydrate result in large bacterial synthesis of nitrogen compounds and so cut out of circulation, at least temporarily, such available forms as ammonia and nitrate. The tendency for carbohydrate and nitrogen to reach a kind of balance in the soil is reflected in the C/N ratio—the lower this is the richer is the soil in general. From recent work by Broadbent and Norman⁹ with tracer N, it appears that the addition of decomposable material to soil may cause the liberation of ammonia from some of the less decomposable nitrogen compounds in the soil—presumably the ligno-protein complex.

Attempts to identify intermediate products between protein and other complex nitrogenous material and inorganic nitrogen in these decompositions have not been remarkably satisfactory. After a considerable range of soils has been examined, we are led to the conclusion that no free amino acids are found in our soils, although many of the common protein amino acids (identified chromatographically) can be produced by HCl hydrolysis; the amount and varieties of these seem to be related to the ecological type in natural soils. Reference should be made in this connexion to the important work of Kojima¹⁰ on muck soils in America.

The most difficult problem in relation to the organic nitrogen of the soil is its relation to the humus fraction. For some time it has been recognized that humus materials, like tans, are able to form fairly stable complexes with protein. Hence in the proximate analysis scheme the resistant humus is called by Waksman "the ligno-protein complex." From early time, attempts have been made to prepare humus fractions free of nitrogen. These have not been generally successful, although material of the same type has been prepared free of nitrogen from brown coal. The question of whether the nitrogen in humus is protein or non-protein, and whether it forms part of the "humus molecule" or not must await future work on the nature of both lignin and humus.

Finally, I wish to refer to some work on what is termed the humus complex. As already indicated, it has been customary in recent times to regard the black-brown constituents of soil organic matter as derived mainly if not wholly from lignin. While it is reasonable to suppose that part of the humus complex

is derived directly from this source, there is much evidence that this is not a complete explanation. It has been found that where organic matter containing known amounts of lignin is added to soil, the rate of increase in humus is greater than the rate of lignin applications. Again, when straw or fresh green material is composted, the dark-coloured products of decomposition may be greater than the amount of lignin present in the original material; and, in fact, dark brown substances having the properties of humus can be extracted from reasonably fresh plant materials by simple extractions which do not de-lignify ligno-cellulose. But the most interesting development in this connexion arises from the investigations of various workers into the pigments of microorganisms—thus, Raistrick¹¹ and his co-workers have found in studies of the pigments of *Penicillia* a yellow substance which they call Fulvic acid, presumably the yellow acid-soluble substance remaining in solution when an alkaline humus extract is acidified. Similarly, Laatsch¹² has produced under culture conditions from actinomycetes (*A. flavochromogenes*) in glucose culture medium a substance which he identifies as a humic acid, although not quite typical of the humus of the soil from which the actinomycete was isolated. It varied from this in its low adsorption value, high N content, and low tanning power, but could be made to resemble humus even in adsorption value by dehydration.

From a fungus (*Stachybotrys alternans*) he has prepared a humus-like substance having the properties of hyalomelanic acid; that is to say, it is soluble in acid alcohol. Thus he has shown that soil microorganisms produce humus substances which can be said to resemble in most respects products obtained from soil. This would seem to be one of the most profitable lines of future investigation of humus, namely, the production by means of organisms isolated from soil, of humus materials in a reasonably pure condition, the establishment of their structural chemistry, and on this basis the separation and identification of these and similar substances from the soil itself.

It is of interest that while, on the whole, bacterial pigments are chiefly carotenoid in structure, fungus pigments are more frequently quinones—hence it is likely that fungi play a greater part in humus-formation than do bacteria. The transformation of carbohydrates into aromatic compounds by fungi is an interesting problem in mycological chemistry, and it seems likely that light might be thrown on the details of this synthesis by studies of the depside structure of the lichen cell-wall.

From these and similar investigations we are justified in concluding that the humus complex of soil, like the carbohydrate, is contributed to by the extra-cellular products of microorganisms. These products being in more intimate contact with the soil and in a more labile condition, are likely to play a greater part in determining the basic structure of the soil than does more massive organic material introduced into the soil. Thus they may be, apart from direct nutritional effects, the chief factor in the organic fertility of soil.

References

- ¹ Jensen, H. R., Proc. Linn. Soc. N.S.W., 1931, LVI, 4, 345
- ² Norman, A. G. and Bartholomew, W. V., Soil Sci., 1943, 56, 143
- ³ Tiurin, I. and Kononova, M., Chem. Social Agric., 1934, 3, (2), 15
- ⁴ Waksman, S. A. and Reuszer, H. W., Soil Sci., 1932, 33, 135
- ⁵ Shewan, J. M., J. Agric. Sci., 1938, 28, 329
- ⁶ Forsyth, W. G. C., Biochem. J., 1947, 41, 176
- ⁷ Forsyth, W. G. C. and Webley, D. M., The Synthesis of Polysaccharides by Bacteria Isolated from Soil. (In press.)
- ⁸ v. Norman, A. G., Proc. Soil Sci. Soc. Amer., 1942, 7, 7
- ⁹ Broadbent, F. E. and Norman, A. G., Proc. Soil Sci. Soc. Amer., 1946, 11, 264
- ¹⁰ Kojima, R. T., Soil Sci., 1947, 64, 157 and 245
- ¹¹ Oxford, A. E., Raistrick, H. and Simonart, P., Biochem. J., 1935, 29, 1102
- ¹² Laatsch, W., Untersuchungen über die Bildung und Anreicherung von Humusstoffen. (Berichte über Landtechnik Heft 4: Verlag H. Neureuter, Wolftrathshausen 1948)